# ab178664 – Thyroxine (Total T4) ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of Thyroxine (Total T4) in Human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 22 August 2016

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## 1. BACKGROUND

Abcam's Thyroxine (Total T4) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of T4 in Human serum and plasma.

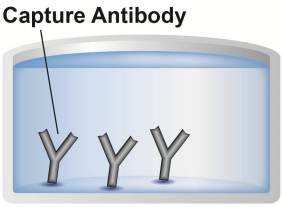
A 96-well plate has been precoated with anti-Thyroxine (T4) antibodies (solid-phase). Samples and the Thyroxine (T4)-HRP conjugate are added to the wells, where Thyroxine (T4) in the sample competes with the added Thyroxine (T4)-HRP conjugate for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Thyroxine (T4) in the sample and the intensity is measured at 450 nm.

The thyroid hormone, Thyroxine (T4) is produced by the thyroid gland. An important component in the synthesis is iodine. The major form of thyroid hormone in the blood is Thyroxine (T4). Thyroxine is converted to the active T3 (three to four times more potent than T4) within cells by deiodinases (5'-iodinase).

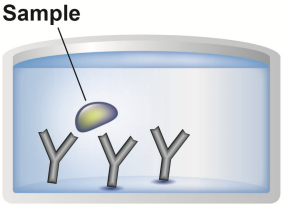
Thyroxine-binding globulin (TGB) is the major carrier protein for circulating thyroid hormone. Only a very small fraction of the circulating hormone is free (unbound) - T4 0.03%.

The thyronines act on the body to increase the basal metabolic rate, affect protein synthesis and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyrotoxicosis or hyperthyroidism is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. Both T3 and T4 are used to treat thyroid hormone deficiency (hypothyroidism).

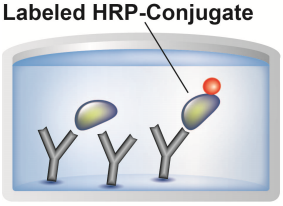
## 2. ASSAY SUMMARY



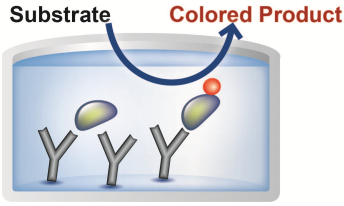

Prepare all reagents, samples, controls and standards as instructed.




Add samples, standards and controls to wells used.




Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.




After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 2-8°C immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

## 5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|---|---|
| Anti-Thyroxine (T4) IgG Coated Microplate (12 x 8 wells) | 96 Wells | 2-8°C |
| Stop Solution | 15 mL | 2-8°C |
| Thyroxine HRP Conjugate | 1.4 mL | 2-8°C |
| TMB Substrate Solution | 15 mL | 2-8°C |
| 50X Washing Solution | 20 mL | 2-8°C |
| Thyroxine Standard 0 - 0 µg/mL | 1 mL | 2-8°C |
| Thyroxine Standard 1 – Concentration lot specific, check label | 1 mL | 2-8°C |
| Thyroxine Standard 2 - 0.05 µg/mL | 1 mL | 2-8°C |
| Thyroxine Standard 3 - 0.10 µg/mL | 1 mL | 2-8°C |
| Thyroxine Standard 4 - 0.15 µg/mL | 1 mL | 2-8°C |
| Thyroxine Standard 5 - 0.25 µg/mL | 1 mL | 2-8°C |
| Conjugate Buffer | 12.5 mL | 2-8°C |
| Strip Holder | 1 unit | 2-8°C |
| Cover Foil | 1 unit | 2-8°C |

## 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 25 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

## 7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

## 8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

## 9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 1,000 mL 1X Washing Solution combine 20 mL 50X Washing Solution with 980 mL deionized water. Mix thoroughly and gently. Diluted solution is stable for 30 days at 2-8°C. In the concentrated solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals.

9.2 **1X Thyroxine HRP Conjugate**

Dilute Thyroxine HRP Conjugate with Conjugate Buffer in a 1:11 ratio. Diluted solution should be used within 24 hours for maximum performance. Store at 2-8°C.

*Note*: prolonged exposure of the conjugate to sunlight may affect the functional characteristics of the assay; therefore do not expose conjugate (and diluted conjugate) to direct sunlight.

9.3 **Thyroxine Standard 1**

Thyroxine Standard 1 concentration is lot specific so always read the label on the vial.

- All other solutions are supplied ready to use

## 10. SAMPLE COLLECTION AND STORAGE

- Collect sample(s) by venipuncture in 10 mL silicone evacuated tube(s). The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation. Use serum or plasma for the T4 procedure. Specimen(s) may be refrigerated at 2-8°C (for a maximum period of 48 hours). If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of –20°C for up to 30 days. When assayed in duplicate, 0.10 mL of the specimen is required.
- The concentration of total thyroxine in serum depends on several factors: the function of the thyroid gland and its regulation, thyroxine binding globulin (TBG) and thyroxine binding to TBG. However, the concentration of total thyroxine alone is not sufficient to monitor the clinical status.
- Total serum thyroxine values may increase during pregnancy or administration of oral contraceptives. The table of drugs and interfering conditions in which the values of total thyroxine are affected have been compiled by the Journal of the American Association of Clinical Chemists.
- Not intended for newborn screening.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants. Do not freeze the solution.
- Maximum precision is required for dispensation of the reagents.

## 11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

## 12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3. Add 25 µL standard, control or sample into their respective wells. Add 100 µL Thyroxine (T4)-HRP Conjugate to each well. Leave a blank well for substrate blank.

12.4. Cover wells with the foil supplied in the kit.

12.5. Incubate for 1 hour at room temperature.

12.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL wash buffer. Avoid overflows from the reaction wells. During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.

12.7. Add 100 µL TMB Substrate Solution into all wells.

12.8. Incubate for exactly 15 minutes at room temperature in the dark.

12.9. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

12.10. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm or against blank within 5 minutes.

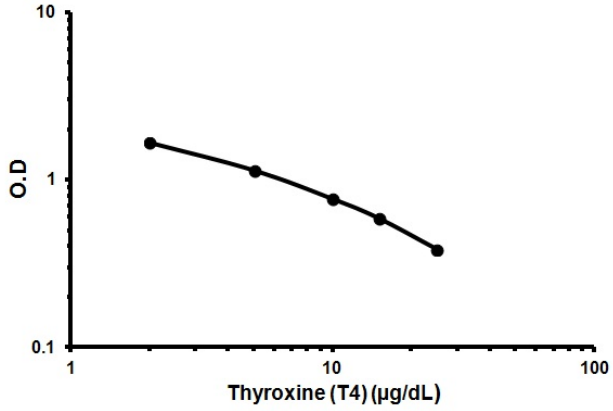
## 13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in μg/mL.

## 14. TYPICAL SAMPLE VALUES

**TYPICAL STANDARD CURVE -**




**REFERENCE VALUES-**

The following values can be used as preliminary guideline until each laboratory has established its own normal range.

| | Mean (µg/mL) | SD | Range (µg/mL) |
|---|---|---|---|
| Value | 0.076 | 0.016 | 0.044 – 0.108 |

**SENSITIVITY –**

The lowest detectable concentration of Thyroxine (T4) that can be distinguished from standard 0 is 0.004 µg/mL at the 95% confidence limit.

**PRECISION –**

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n= | 20 | 10 |
| %CV | ≤ 8.16% | ≤ 8.42% |

**RECOVERY –**

The recovery of 12.5 – 25 – 50 – 100 µg/mL Thyroxine (T4) added to sample gave an average value (±SE) of 97.8% ± 5% with reference to the original concentrations.

## 15. ASSAY SPECIFICITY

The cross reactivity of the Thyroxine (T4) antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross reactivity was calculated by deriving a ratio between doses of interfering substance to dose of Thyroxine (T4) needed to displace the same amount of tracer:

| Substance | Cross Reactivity | Concentration |
|---|---|---|
| l-Thyroxine | 1.0000 | - |
| d-Thyroxine | < 0.9800 | 10µg/ml |
| Trylodothyronine | < 0.0300 | 100µg/ml |
| d-Tryiodothyronine | < 0.0150 | 100µg/ml |
| Iodothyrosine | < 0.001 | 100µg/ml |
| Trilodotetraacetic acid | < 0.0001 | 100µg/ml |
| Di-Iodotetraacetic acid | < 0.0001 | 100µg/ml |

## 16. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| Low signal | Incubation time to short | Try overnight incubation at 4 °C |
| | Precipitate can form in wells upon substrate addition when concentration of target is too high | Increase dilution factor of sample |
| | Using incompatible sample type (e.g. serum vs. cell extract) | Detection may be reduced or absent in untested sample types |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution |
| Large CV | Bubbles in wells | Ensure no bubbles present prior to reading plate |
| | All wells not washed equally/thoroughly | Check that all ports of plate washer are unobstructed/wash wells as recommended |
| | Incomplete reagent mixing | Ensure all reagents/master mixes are mixed thoroughly |
| | Inconsistent pipetting | Use calibrated pipettes & ensure accurate pipetting |
| | Inconsistent sample preparation or storage | Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles) |

| Problem | Cause | Solution |
|---|---|---|
| High background | Wells are insufficiently washed | Wash wells as per protocol recommendations |
| | Contaminated wash buffer | Make fresh wash buffer |
| | Waiting too long to read plate after adding stop solution | Read plate immediately after adding stop solution |
| Low sensitivity | Improper storage of ELISA kit | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
| | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

## 17. NOTES

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